TECHNICAL MANUSCRIPT 312

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DEPARTMENT OF THE ARMY
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Herman Rutner

George Svarnas

Physical Science Division
BIOLOGICAL SCIENCES LABORATORY

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FOREWORD

This synthesis was patented under U.S. Patent 3,150,124, dated 22 September 1964.

ABSTRACT

Kinetin riboside is of interest in plant physiology as a mitotic stimulant and in the tissue culture of cancer cells for its unusual differential toxicity toward adult human fibroblasts in the presence of human carcinoma cells. Three multi-step methods for its synthesis have been reported.

This report describes a simple two-step synthesis for kinetin riboside that, unlike the cited methods of preparation, utilizes intermediates that are readily available and requires only a fraction as much time and effort for completion.

Authentic kinetin riboside and material prepared by this synthesis were subjected to a number of physical and chemical tests and the data thus obtained are compared.

I. INTRODUCTION

Kinetin riboside (6-furfurylamino-9-β-D-ribofuranosyl purine, IV) is of interest in plant physiology as a mitotic stimulant[1]* and in the tissue culture of cancer cells for its unusual differential toxicity toward adult human fibroblasts in the presence of human carcinoma cells.[2] Three methods for its synthesis have been reported.

Kissman and Weiss[3] reacted 2,3,5-tri-O-benzoyl-D-ribofuranosyl chloride[4] with the chloromercuri derivative of 6-chloro-purine.[5] The resulting 6-chloro-9-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl) purine was purified by chromatography on alumina. The purified material was then converted to the 6-furfurylamino derivative and debenzoylated to yield IV in an over-all yield of 22% based on D-ribose.

Hampton et al.[2] condensed the chloromercuri derivative of 6-methylmercaptopurine[6] with 2,3,5-tri-O-acetyl-D-ribofuranosyl chloride[7] to form 6-methylmercapto-9-(2,3,5-tri-O-acetyl-D-ribofuranosyl) purine. Deacetylation followed by reaction with furfurylamine yielded IV in 10% yield based on D-ribose.

Brug** obtained IV by treating the chloromercuri derivative of 6-N-acetyl-furfurylaminopurine with 2,3,5-tri-O-benzoyl-D-ribofuranosyl chloride, followed by deacylation of the product so formed.

Each of these methods requires the use of intermediates that are not readily available and must, therefore, be prepared as a necessary preliminary to the synthesis of IV.

This report describes a synthesis of IV that was developed as an alternative to the foregoing multi-step methods. It is an extension of the work of Bullock et al.,[8] in which the preparation of kinetin (6-furfurylaminopurine) from adenine in two steps is reported. In our procedure adenosine (II) is converted to an uncharacterized intermediate presumed to be 6-furoylamino-9-(2,3,5-tri-O-furoyl-β-D-ribofuranosyl) purine (III) by reaction with 2-furoyl chloride (I) in anhydrous pyridine. Reduction of III with lithium aluminum hydride in diethylene glycol dimethyl ether gives IV in 11.4% yield. This simple two-step synthesis (Figure 1), unlike the cited methods of preparation, utilizes intermediates that are readily available and requires only a fraction as much time and effort for completion.

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* Page 125.

** Unpublished data of J. Brug, referenced on p. 148 of Strong.[1]

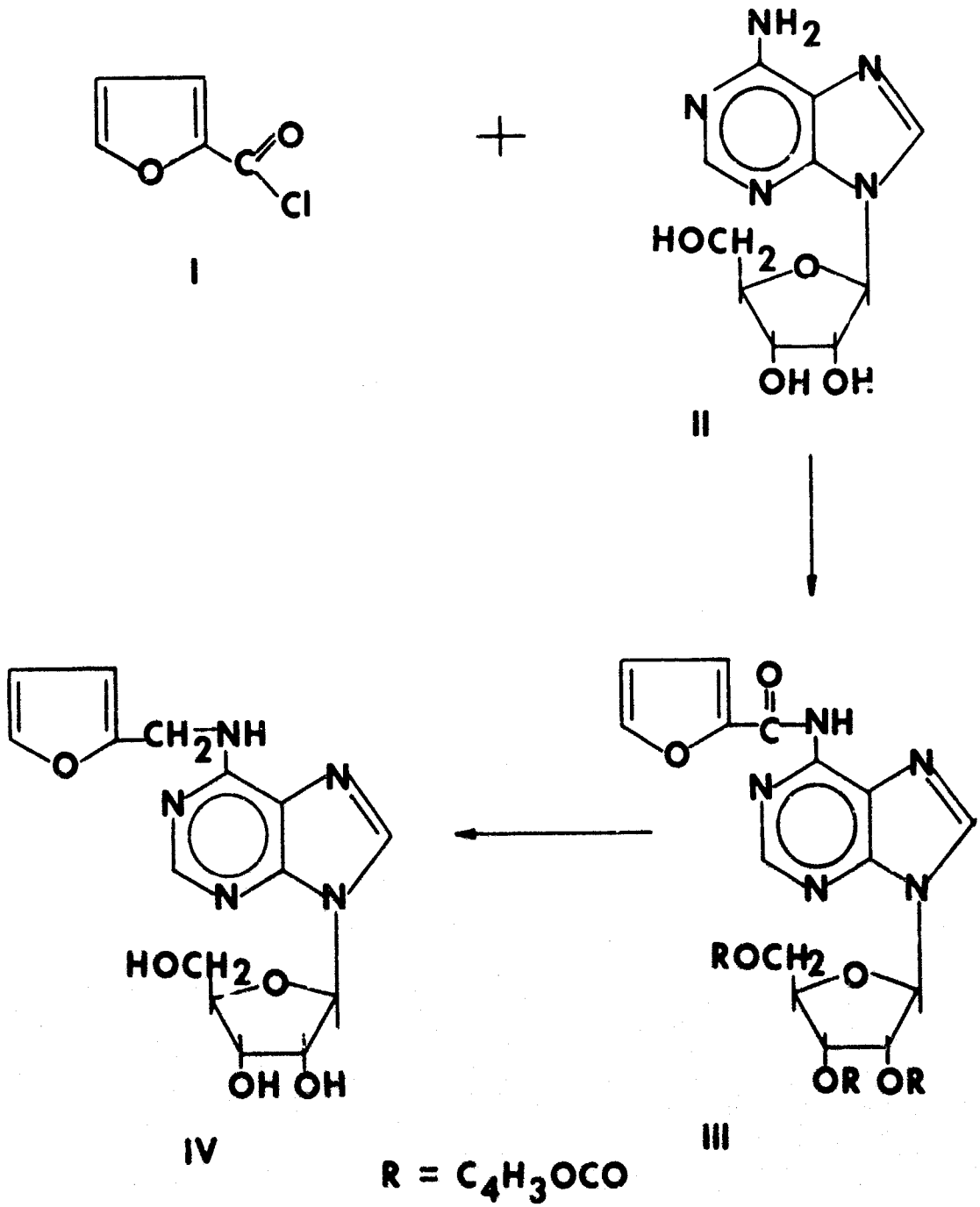

Figure 1. Two-Step Synthesis of Kinetin Riboside.

II. DISCUSSION

2-Furoyl chloride was reacted with adenosine in varying molar ratios and under a variety of reaction conditions. This treatment yielded a crude product in which it was possible to demonstrate the presence of one to three components when samples were chromatographed* and the chromatograms examined under UV excitation. The number of components obtained appeared to be dependent not only on the reaction parameters, but also upon the method of recovery of the crude product from the reaction mixture. No definitive attempt was made to characterize these components, but it is believed that they are furoyl derivatives of adenosine representing three degrees of furoylation up to and possibly including the pentafuroyl derivative. The possibility of the existence of the pentafuroyl derivative is inferred from the reported[9,10] formation of a pentabenzoyl derivative when adenosine is benzoylated by analogous procedures.

The multi-component amorphous reaction product resulting from the furoylation was treated with lithium aluminum hydride without further resolution.

Reduction of crude III with lithium aluminum hydride in a suitable inert solvent** was found fortuitously to effect the two chemical conversions required to yield IV during the course of a single treatment. One involves the conversion of the carbonyl group of the furoylamino side-chain to a methylene group. The other effects the reductive removal of the furoyl groups from the carbohydrate residue with the regeneration of the original moiety.

Table 1 lists several physical constants for kinetin riboside prepared by our method and includes the corresponding cited literature values. Three other criteria further confirmed that the products obtained by the three procedures are identical: (i) The infrared spectra of all three products referred to in footnote (b), Table 1, were determined by the KBr pellet method on a Perkin-Elmer (Model 21) recording infrared spectrophotometer and were found to be identical. (ii) Samples of our product and authentic kinetin riboside*** were recrystallized from methanol and their respective melting points, together with a mixed melting point, were obtained immediately after recrystallization. The melting characteristics in all three determinations were identical, with no depression observed in the mixed melting point determination. (iii) Samples of kinetin riboside prepared by our method and by the method of Kissman and Weiss in our laboratory were hydrolyzed in acid for approximately 4 hours, and the chromatographic characteristics of samples of the hydrolysates taken at three time intervals during the hydrolysis were determined. On the basis of this parameter, the materials were identical.

* System: n-butanol: water, 86:14; paper: Whatman No. 1, used in the descending mode.

** Diethylene glycol dimethyl ether, tetrahydrofuran, and ether-dioxane (5:2) were each successfully used as solvent systems.

*** Kindly furnished by Dr. G.B. Brown, Sloan-Kettering Institute for Cancer Research, New York, N.Y.

TABLE 1. COMPARISON OF KINETIN RIBOSIDE PREPARED BY THREE DIFFERENT METHODS

Method of Preparation	Melting Point, C	Optical Rotation in Ethanol, $(\alpha)_D^{25}$	R_f [b] (System:n-butanol-water, 86:14)	UV Spectra	
				λ_{max}^{EtOH}	ϵ
This report	151-153[a]	-63.5° (C=1.09)	0.73	267	19,800
Kissman and Weiss	148-150[c]	-53.5° (C=1.13)[c]	0.73	268[c]	19,000[c]
Hampton, et al.	151-152[c]	-	0.72[b]	267[c]	19,300[c]

a. Corrected value, determined on the Kofler M.P. apparatus.
b. Values obtained when our product, material prepared in our laboratory by the method of Kissman and Weiss, and authentic kinetin riboside obtained from G.B. Brown were chromatographed on the same sheet of paper.
c. Value reported by indicated authors.

III. EXPERIMENTAL PROCEDURES

A. FUROYLATION STEP

Adenosine (1.95 grams, 7.3 mmoles), dried 2 hours at 60 C in vacuo, was suspended in 25 ml of dry pyridine. 2-Furoyl chloride (10 ml, 70.0 mmoles) was added and the mixture was refluxed for 30 minutes. The reaction mixture was cooled and the solvent and excess 2-furoyl chloride were removed in vacuo. The heavy residual syrup was dissolved in 75 ml of chloroform and the solution was washed successively with 25 ml of water, five 25-ml portions of 0.1 M hydrochloric acid, 25 ml of saturated aqueous sodium bicarbonate solution, and two 25-ml portions of water. The organic phase was treated for 15 minutes with a mixture of 5 grams of anhydrous magnesium sulfate and 2 grams of Norit, filtered, and the residue washed with 50 ml of chloroform. The washings and the filtrate were combined and concentrated at room temperature in vacuo to 25 ml, 4.0 grams of silicic acid (100 mesh) was added, and the mixture was heated for 5 minutes and filtered. The silicic acid was washed with 30 ml of hot chloroform and the filtrate and washings were combined and evaporated to a heavy syrup in vacuo. The syrup was extracted with two 25-ml portions of warm ether and the residue dried in vacuo, yielding 4.7 grams of a buff-colored fluffy solid. This material was subjected to reductive treatment without further purification.

B. REDUCTION STEP

To 25 ml of redistilled diethylene glycol dimethyl ether in a 200-ml three-necked round-bottom flask equipped with an addition funnel, stirrer, and drying tube was added 2.25 grams (59.0 mmoles) of lithium aluminum hydride. To the stirred mixture was added 4.5 g (6.99 mmoles) of crude 6-furoylamino-9-(tri-O-furoyl-β-D-ribofuranosyl) purine dissolved in 25 ml of diethylene glycol dimethyl ether over a period of 30 minutes (slight exothermic reaction). After the addition was complete, the reaction mixture was heated at approximately 50 C for 30 minutes. The mixture was cooled and 10 ml of ethyl acetate was added, followed by a drop-wise addition of 10 ml of water with stirring over a period of 10 minutes. The pH was then immediately adjusted to 6 to 7 with concentrated hydrochloric acid, the mixture was filtered and the solid washed with 50 ml of warm methanol. The filtrate and washings were combined and concentrated in vacuo with the aid of a water-bath at a temperature held below 65 C. The heavy residual syrup was taken up in 25 ml of absolute methanol and refrigerated overnight. The resulting crystals were collected on a filter and washed with a small amount of chilled methanol. Recrystallization from acetone-petroleum ether (bp 30 to 75 C) yielded 0.29 grams (11.4%, based on adenosine) of white crystalline product, mp 151 to 153 C (sinters below mp).

C. ACID HYDROLYSIS OF KINETIN RIBOSIDES

Two samples of kinetin riboside, one prepared by our method and the other prepared by the method of Kissman and Weiss in our laboratory, were hydrolyzed in 0.1 M hydrochloric acid. Samples of each hydrolysate were taken at 110, 170, and 230 minutes and spotted on Whatman No. 1 paper. The spots were neutralized with a pyridine-acetone solution and the paper chromatographed in the previously cited system.*

Two spots each were observed for the 110-minute samples: (a) a UV-absorbing spot, R_f 0.81, and (b) a spot visualized by a carbohydrate spray reagent,** R_f 0.17. Evidence was obtained suggesting that spots (a) and (b) are kinetin and D-ribose respectively by repeating the chromatography, using samples of the authentic compounds as reference markers.

The 170- and 230-minute samples exhibited, in addition to spots (a) and (b), a second UV-absorbing spot, R_f 0.30. This substance was not identified.

These studies indicated that the hydrolysis products of the two original samples are identical not only in the number of components demonstrable by paper chromatography, but also with respect to the size, shape, relative intensity, and R_f values of the spots, which are characteristic of each of these components. These data, therefore, further confirm that the original samples are identical.

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* System: n-butanol: water, 86:14; paper: Whatman No. 1, used in the descending mode.

** 0.. M Silver nitrate and 5 M ammonium hydroxide, 1:1; after drying, the chromatogram was developed at 100 C.

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